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*Environmental Toxicology*TOXICITY AND BIOACCUMULATION OF SEDIMENT-ASSOCIATED
CONTAMINANTS USING FRESHWATER INVERTEBRATES:
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Abstract—This paper reviews recent developments in methods for evaluating the toxicity and bioaccumulation of contaminants associated with freshwater sediments and summarizes example case studies demonstrating the application of these methods. Over the past decade, research has emphasized development of more specific testing procedures for conducting 10-d toxicity tests with the amphipod *Hyaella azteca* and the midge *Chironomus tentans*. Toxicity endpoints measured in these tests are survival for *H. azteca* and survival and growth for *C. tentans*. Guidance has also been developed for conducting 28-d bioaccumulation tests with the oligochaete *Lumbriculus variegatus*, including determination of bioaccumulation kinetics for different compound classes. These methods have been applied to a variety of sediments to address issues ranging from site assessments to bioavailability of organic and inorganic contaminants using field-collected and laboratory-spiked samples. Survival and growth of controls routinely meet or exceed test acceptability criteria. Results of laboratory bioaccumulation studies with *L. variegatus* have been confirmed with comparisons to residues (PCBs, PAHs, DDT) present from synoptically collected field populations of oligochaetes. Additional method development is currently underway to develop chronic toxicity tests and to provide additional data-confirming responses observed in laboratory sediment tests with natural benthic populations.

Keywords—Toxicity Bioaccumulation *Hyaella azteca* *Chironomus tentans* *Lumbriculus variegatus*

INTRODUCTION

Sediment provides habitat for many aquatic organisms and is a major repository for many of the more persistent chemicals that are introduced into surface waters. Over the past 10 years, increasing interest has focused on developing and applying methods for assessing the severity of contaminated sediments. This paper reviews recent developments in methods for evaluating the toxicity [1-10] and bioaccumulation [1,11,12] of contaminants associated with freshwater sediments and summarizes example case studies demonstrating the application of these methods.

Nebeker et al. [5] first described general methods for conducting toxicity tests with freshwater sediments using amphipods, midges, mayflies, and cladocerans. This initial publication stimulated research on developing more detailed testing procedures. This paper outlines specific methods recently described for conducting toxicity tests with *Hyaella azteca* and *Chironomus tentans* [1,2] and describes general methods for conducting bioaccumulation tests with *Lumbriculus variegatus* [1,11]. These test organisms were selected for

method development after a variety of potential test organisms were considered (Tables 1 and 2). Numerous investigators have successfully evaluated the toxicity of contaminated sediments with *H. azteca* (e.g., [5-7,9,13-20]) and *C. tentans* (e.g., [5,9,21-28]). *Hyaella azteca* has also been used to evaluate the toxicity of estuarine sediments (up to 15‰ salinity; [1,2]). A limited number of investigators have also presented general guidance for the use of *L. variegatus* to examine bioaccumulation of chemicals from sediment [1,12,29-33].

Selection of test organisms

The choice of a test organism has a major influence on the relevance, success, and interpretation of a test [2,34]. Table 1 outlines important criteria that should be considered when selecting organisms for toxicity testing with sediments. The amphipod *H. azteca* and the midge *C. tentans* were selected for method development [1-4] from this list of test organisms because of their ease of culture in the laboratory [7], contact with sediment, tolerance to varying sediment physicochemical characteristics [7,28,35], interlaboratory comparisons of toxicity responses [1,2], existing data on the relative sensitivity to contaminants (Table 3; [14,19,21,36,37]), and data confirming the response of laboratory test organisms with natural benthic populations [19-23,38-41]. The additional organisms listed in Table 1 that might be appropriate

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Table 1. Selection criteria for sediment toxicity test organisms [1,2]

Criterion	<i>Hyaella azteca</i>	<i>Diporeia</i> spp.	<i>Chironomus tentans</i>	<i>Chironomus riparius</i>	<i>Lumbriculus variegatus</i>	<i>Tubifex tubifex</i>	<i>Hexagenia</i> spp.	Mollusks	<i>Daphnia</i> spp. or <i>Ceriodaphnia</i> spp.
Relative sensitivity toxicity database	+	-	+	-	+	-	-	-	-
Round-robin testing	+	-	+	-	+	-	-	-	-
Contact with sediment	+	-	+	-	+	-	-	-	-
Laboratory culture	+	-	+	+	+	+	+	+	+
Taxonomic identification	+	+/ -	+/ -	+/ -	+	+	+	+	+
Ecological importance	+	+	+	+	+	+	+	+	+
Geographic distribution	+	+/ -	+	+	+	+	+	+	+/ -
Sediment physicochemical tolerance	+	+	+/ -	+	+	+	-	+	NA
Response confirmed with benthic populations	+	+	+	+	+	+	+	-	+
Peer reviewed	+	+	+	+	+	+	+	-	+/ -
End points monitored	S,G,M	S,B,A	S,G,E	S,G,E	B,S	S,R	S,G	B	S,G,R

A "+" or "-" rating indicates a positive or negative attribute; NA, not applicable.

S = survival, G = growth, B = bioaccumulation, A = avoidance, R = reproduction, M = maturation, E = emergence.

for toxicity testing with sediments do not now meet all of these selection criteria because, historically, little emphasis has been placed on method development. The reader is encouraged to consult other documents for description of procedures for testing these additional species listed in Table 1 [2,4].

Various organisms have been suggested for use in studies of chemical bioaccumulation from aquatic sediments (Table 2, [8,42]). The following criteria outlined in Table 2 were used to select *L. variegatus* for bioaccumulation method development: (a) ease of culture and handling [12], (b) known chemical exposure history, (c) adequate tissue mass for chemical analyses, (d) tolerance of a wide range of sediment physicochemical characteristics [28], (e) low sensitivity to contaminants associated with sediment (Table 3), (f) amenability to long-term exposures without feeding [12], (g) ability to accurately reflect concentrations of contaminants in field-exposed organisms (i.e., exposure is realistic), and (h) data confirming the response of laboratory test organisms with natural benthic populations [1,2,43]. Thus far, extensive interlaboratory testing has not been conducted with *L. variegatus* [1]. Other organisms did not meet many of these selection criteria outlined in Table 2 including mollusks (valve closure), midges (short life cycle), mayflies (difficult to culture), *H. azteca* (small tissue mass, too sensitive), cladocerans and fish (not in direct contact with sediment).

METHODS

The following section describes methods for culturing organisms, experimental design, collecting and handling sediments, general testing procedures, and quality assurance/quality control (Appendices A and B). Use of the specific test methods described in U.S. Environmental Protection Agency (EPA) [1] and in American Society for Testing and Materials (ASTM) [2,11] method procedures increases comparability between tests (i.e., must and should statements [2,11]); however, data obtained using modified versions of these methods might provide useful information on new procedures for conducting sediment tests [2-4].

Culturing procedures and performance-based criteria

The Environmental Monitoring Management Council (EMMC) of the EPA recommends use of performance-based methods in developing chemical analytical procedures [44]. Performance-based methods are defined by the EMMC as a monitoring approach that permits use of appropriate methods meeting established performance criteria. Many biological methods now recommend the use of performance-based criteria as the approach through which individual laboratories can evaluate culture methods [1-4,11], in contrast, control-based criteria only describe one method for culturing test organisms [e.g., 45]. A performance-based approach allows each laboratory to use unique culturing procedures as long as test organisms meet test acceptability criteria outlined in Appendix B.

Although various procedures for culturing test organisms are described in methods manuals developed by the EPA [1] and ASTM [2,11], the procedure selected to culture *H. azteca* must produce 7- to 14-d-old amphipods to start a sediment

Table 2. Selection criteria for sediment bioaccumulation test organisms [1,8,11,12,42]

Criterion	<i>Lumbriculus variegatus</i>	Mollusks	Midges	Mayflies	Amphipods	Cladocerans	Fish
Laboratory culture	+	—	+	—	+	+	+
Known chemical exposure	+	—	+	+/-	+	+	+
Adequate tissue mass	+/-	+	—	+	—	—	+
Low sensitivity to contaminants	+	+	—	—	—	—	+/-
Feeding not required during testing	+	+	—	+	—	—	+
Realistic exposure	+	+/-	+	+	+	—	—
Sediment physicochemical tolerance	+	?	+/-	—	+	NA	NA
Response confirmed with benthic populations	+	?	?	?	+	?	—

A "+" or "-" rating indicates a positive or negative attribute; NA, not applicable; ?, unknown.

test (Appendix B). The procedure selected to culture *C. tentans* must produce 3rd instar larvae to start a test (at least 50% of the larvae must be 3rd instar and the remaining larvae must be 2nd instar; Appendix B). The procedure selected to culture *L. variegatus* should produce relatively large quantities of adult organisms necessary for bioaccumulation testing.

Experimental design

Decisions concerning various aspects of experimental design, such as sampling, number of treatments, and the number of replicates/treatment, are based on the purpose of the test and the methods of data analyses [1-4,11]. For example, if the purpose of the study is to conduct a reconnaissance field survey to identify contaminated sites for further investigation, one sample from each site allows for sampling a larger area [2]. The lack of replication at a site usually precludes statistical comparisons (e.g., ANOVA), but these surveys can be used to identify contaminated sites for further study or may be evaluated using regression techniques. If the purpose of the study is to conduct a quantitative sediment survey, field replicates (separate samples from different grabs collected at the same site) would need to be collected from each site. Chemical and physical characterizations of each replicate grab would be required for toxicity or bioaccumulation testing. Separate subsamples might be used to deter-

mine within-sample variability or for comparisons of test procedures (e.g., comparative sensitivity among test organisms), but these subsamples cannot be considered true field replicates for statistical comparisons among sites.

When testing hypotheses, the minimum significant difference is inversely proportional to the number of replicates. Because no consensus exists for criteria to establish a biologically acceptable difference, the appropriate minimum significant difference is a data quality objective established by the user based on data requirements, the logistics and economics of test design, and the ultimate use of the data. Based on previous experience, eight replicates/treatment for toxicity tests and five replicates/treatment for bioaccumulation tests are recommended for routine testing [1-4,11]. The minimum number of replicates/treatment that should be tested is four, but as many replicates as is economically and logistically possible should be included.

Statistical testing of hypotheses can be designed to control the chances of making incorrect decisions (Type I or II errors). Type I (α) errors result in the incorrect conclusion that a sample is contaminated (toxic) when it is not contaminated (false positive). Type II (β) errors result in the incorrect conclusion that a sample is not contaminated when it is contaminated (false negative). Values for α traditionally range from 0.1 to 0.01, with 0.05 (or 5%) used most commonly. Historically, having chosen α , environmental researchers have ignored β and the associated power of the test ($1-\beta$). The consequences of a Type II error in environmental studies may be the most important criteria to consider in experimental designs and data analyses of environmental issues. Critical components of experimental design associated with hypothesis testing are (a) the required minimum detectable difference between the treatment and control or reference responses, (b) the variance among treatment and control replicates, (c) the number of replicates for the treatment and control samples, and (d) the selected probabilities of Type I (α) and Type II (β) errors.

Sediment collection and handling

Detailed information on health and safety, facilities and equipment, sources of water and formulated sediment, and sediment collection, storage, manipulation, and characterization is described in methods manuals developed by the EPA [1], ASTM [2,11], and Environment Canada (EC) [3,4]. Be-

Table 3. Water-only, 10-d LC50 ($\mu\text{g/L}$) values for *Hyaella azteca*, *Chironomus tentans*, and *Lumbriculus variegatus* for chemicals tested in soft water (hardness 40 mg/L as CaCO_3 ; [37])

Chemical	<i>H. azteca</i>	<i>C. tentans</i>	<i>L. variegatus</i>
Copper	35	54	35
Zinc	73	1,125 ^a	2,984
Cadmium	2.8 ^b	NT ^c	158
Nickel	780	NT	12,160
Lead	<16	NT	794
<i>p,p'</i> -DDT	0.07	1.23	NT
<i>p,p'</i> -DDD	0.17	0.18	NT
<i>p,p'</i> -DDE	1.39	3.0	>3.3
Dieldrin	7.6	1.1	NT
Chlorpyrifos	0.086	0.07	NT

^a50% mortality at highest concentration tested.

^b70% mortality at lowest concentration tested.

^cNT, not tested.

fore preparation or collection of sediment, procedures should be established for safe handling and disposal of sediments that might contain unknown quantities of toxic contaminants.

Sediment should be collected with as little disruption as possible; however, subsampling, compositing, or homogenization of sediment samples may be required for some experimental designs. Sediment should be collected from a depth that will represent expected exposure. For example, most oligochaetes inhabit only the upper 2 cm of sediment but may burrow 4 to 15 cm. Given that contaminants of concern and influencing sediment characteristics are not always known a priori, sediments should be held in the dark at 4°C and tests should be started as soon after collection as possible. Recommended sediment holding time ranges from less than 2 [34] to less than 8 weeks [46].

Sediment tends to settle during shipment; therefore, overlying water should be mixed back into the sediment during homogenization. Sediment samples should not be sieved to remove indigenous organisms, although large indigenous organisms and large debris can be physically removed (e.g., using forceps). If sediments are sieved to remove indigenous organisms [47], the influence of sieving on the subsequent concentrations of contaminants should be documented.

Test sediments can be generated by spiking control sediments with chemical contaminants. Consistent spiking procedures should be followed to allow interlaboratory comparisons. Organic carrier solvents should be avoided because of potential changes to the dissolved organic carbon concentration in pore water. Spiked sediment should be aged at least 1 month before starting a test; however, equilibration for some chemicals may require even longer periods [1]. Some chemicals such as pesticides may enter sediment in a pulse; hence equilibration of spiked sediment may not be appropriate. See the methods manual developed by ASTM [48] for additional detail regarding spiking methods.

General testing procedures

Each test chamber receives two volume additions/d of overlying water using either automated or manual systems (Appendix A; [49,50]). Automated systems require more equipment and initial construction time, but manual addition of water takes more time during a test. In addition, automated systems generally result in less suspension of sediment compared with manual renewal of water. Water-delivery systems are calibrated before a test is started to ensure the systems are functioning properly. At any particular time during the test, flow rates through any two test chambers should not differ by more than 10%, and hardness, alkalinity, pH, and ammonia concentrations in the water above the sediment within a treatment should not vary by more than 50% during the test. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. For site-specific evaluations, characteristics of the overlying water should be as similar as possible to the site where sediment is collected.

A test begins when the organisms are added to the test chambers (day 0). The day before the sediment test is started (day -1) each sediment sample is typically thoroughly mixed, visually inspected to judge homogeneity, and added to the test chambers. If a quantitative measure of homogeneity is

required, replicate subsamples can be taken and analyzed for parameters such as total organic carbon, chemical concentration, or grain size. Each test chamber should contain the same amount of sediment, determined either by volume or weight. Overlying water is added on day -1 in a manner that minimizes suspension of sediment.

A subsistence diet is provided during toxicity tests because without addition of food, *H. azteca* and *C. tentans* may starve during 10-d exposures [28]. *Lumbriculus variegatus* does not require the addition of food during bioaccumulation tests (Appendix A). Although the addition of the food may alter the availability of the contaminants in the sediment [51,52], a "worst case" change in total organic carbon of about 0.1% would occur at the recommended feeding rates [9]. The amount of food added to the test chambers has been kept to be minimum to prevent excessive fungal or bacterial growth from developing on the sediment surface.

Toxicity tests

The 10-d test methods with *H. azteca* and *C. tentans* are conducted at 23°C with a 16:8 h light:dark photoperiod at an illuminance of about 500 to 1,000 lux (Appendix A). Test chambers are 300-ml beakers containing 100 ml of sediment and 175 ml of overlying water. Eight replicates, each containing ten 7- to 14-d-old amphipods or ten 3rd-instar midges are recommended for routine testing. Each chamber receives a daily ration of 1.5 ml of yeast-cerophyl-trout chow (YCT) in the amphipod test or 1.5 ml of a 4 g/L Tetrafin® suspension in the midge test. Average survival in the control sediment must be at least 80% for *H. azteca* or 70% for *C. tentans*, and average dry weight of individual *C. tentans* must be at least 0.6 mg at the end of the test. Additional requirements for test acceptability are listed in Appendix B. The method for *C. tentans* has been used to test 2nd instar larvae of *Chironomus riparius* [2,4].

Survival is the primary endpoint measured at the end of the 10-d test with *H. azteca*. Growth (either dry weight or length) is an optional measurement but may be a more sensitive toxicity endpoint [3,18,39]. The duration of the 10-d test started with 7- to 14-d-old amphipods is not long enough to determine sexual maturation or reproductive effects. Because of their size, quantitative recovery of young amphipods (e.g., 1- to 7-d old) from sediment is more difficult relative to recovery of older and larger amphipods (e.g., 14- to 21-d old; [10]). To facilitate recovery at the end of the test, 10-d tests are started with 7- to 14-d-old amphipods. As toxicant sensitivity of *H. azteca* is relatively similar at ages <1- to 26-d old [53], starting tests with 7- to 14-d-old amphipods would not bias the results of a test. Environment Canada [3] recommends conducting *H. azteca* tests starting with 2- to 9-d-old organisms and monitoring both survival and growth after 14 d of exposure.

Dry weight and survival are the primary endpoints measured at the end of the 10-d test with *C. tentans* [1,2,4]. A 10-d test starting with 3rd-instar larvae is not long enough to determine emergence of adult midges. If length or head capsule width is to be determined, additional replicate beakers should be set up to sample midges at the end of an exposure. Although 3rd-instar midges may not be as sensitive as younger organisms [39], the larger larvae are recom-

mended for use because they are easier to handle and to isolate from sediment at the end of a test.

Hyalella azteca tolerate natural and formulated sediments with a wide range of grain size and organic matter [7,28,35]. Although survival or growth of *C. tentans* was not reduced over a wide range in sediment grain sizes in 10-d tests with formulated sediment, survival was reduced in sediments with less than 0.91% organic matter without the addition of food [35]. Also, even though grain size and organic carbon were not significantly correlated with the survival of *C. tentans* in toxicity tests in which organisms were fed, linear modeling indicated that growth increased in coarser sediment [28].

Indigenous organisms can influence the response of test organisms in sediment through predation [7], competition for nutrients, or alteration of exposure conditions (e.g., oligochaetes in the presence of amphipods or midges; [47]). However, the ability to observe these alterations will depend on the number of predators and competing organisms in the test sediment. Thus, the number and biomass of indigenous organisms in field-collected sediment should be determined.

Bioaccumulation tests

The 28-d bioaccumulation test with *L. variegatus* is conducted with adult oligochaetes at 23°C with a 16:8 h light:dark photoperiod at an illuminance of about 500 to 1,000 lux (Appendix A). Test chamber size reportedly ranges from 4 to 6 L and contains 1 to 2 L of sediment and 1 to 4 L of overlying water with five replicates recommended for routine testing. To minimize depletion of sediment contaminants, a ratio of 50:1 total organic carbon in sediment to dry weight of organisms is recommended. A minimum of 1 g (wet weight)/replicate, with up to 5 g/replicate, should be tested. Requirements for test acceptability are outlined in Appendix B.

If sediments could be toxic to *L. variegatus*, a 4-d toxicity screening test should be conducted before starting a bioaccumulation test [1,11]. Toxicity test chambers are 300-ml beakers containing 100 ml of sediment and 175 ml of overlying water. Four replicates, each containing 10 adult oligochaetes/replicate, are recommended for routine testing [12]. Endpoints monitored at the end of a toxicity test are number of organisms and behavior. Numbers of *L. variegatus* in the toxicity screening test should not be significantly reduced in the test sediment relative to the control sediment. Test organisms should burrow into test sediment because avoidance of test sediment by *L. variegatus* may reduce bioaccumulation.

At the end of a bioaccumulation test, live oligochaetes are transferred to a 1-L beaker containing overlying water without sediment for 24 h to eliminate gut contents (oligochaetes clear more than 90% of the gut contents in 24 h [31]). A correction for the extent of elimination from the body burden may need to be made for compounds with log K_{ow} less than 5 [54]. Oligochaetes are not placed in clean sediment to eliminate gut contents because clean sediment can contribute 15 to 20% to the dry weight of the oligochaetes, resulting in a dilution of contaminant concentrations on a dry-weight basis. Minimum tissue mass required for various analyses at selected lower limits of detection has been provided in methods manuals developed by the EPA [1] and ASTM [11]. Depending on study objectives, total lipids can be measured on a sub-

sample of the total tissue mass of each replicate sample. Dry weight of oligochaetes can be determined on a separate subsample from each replicate.

Field-collected sediments may include indigenous oligochaetes. Fortunately, both the behavior and the appearance of indigenous oligochaetes are usually different from *L. variegatus*. To check for the presence of indigenous oligochaetes in field-collected sediment, extra chambers without the addition of *L. variegatus* can be tested [12]. Native oligochaetes in a laboratory study were shown to alter bioaccumulation [55].

Because bioaccumulation tests are often used in ecological or human health risk assessments, the procedures are designed to generate estimates of steady-state tissue residues. Eighty percent of steady-state is used as the general goal for a test [11]. An option when conducting a bioaccumulation test is to perform a kinetic study to estimate steady-state concentrations instead of conducting a 28-d bioaccumulation test (e.g., sample on days 1, 3, 7, 14, 28; [1,11]). A kinetic test can be used when 80% of steady-state will not be obtained within 28 d or when more precise estimates of steady-state tissue residues are required.

Quality assurance and quality control

The ability of a laboratory to complete a test successfully will improve with experience. A program for certification of laboratories with the methods outlined in Appendix A has not yet been established. Before a laboratory routinely conducts sediment tests, intralaboratory precision should be evaluated by performing five or more 96-h water-only reference toxicity tests. Procedures for judging the acceptability of reference toxicity tests have been outlined [1-4,11]. A laboratory also should demonstrate the ability to perform tests by conducting five exposures with a control sediment. Ideally, these sediment exposures would be conducted concurrently with the five reference toxicity tests. Survival and growth of organisms in these whole-sediment exposures will demonstrate whether the facilities, water, control sediment, and handling techniques are adequate to ensure acceptable survival of organisms in the control sediment (Appendix B). Evaluations may also be made on the magnitude of the within-chamber and between-chamber variability. Laboratories should also demonstrate that their personnel are able to recover an average of at least 90% of the organisms from whole sediment. For example, test organisms could be added to control or test sediment and recovery could be evaluated after 1 h. Results of these amphipod recovery evaluations could be used to separate effects on survival from the inability to recover small organisms such as *H. azteca* from sediment. Moreover, recovery of preserved amphipods from an externally supplied "amphipod-spiked" sediment could be an additional measure of quality control for sediment tests with *H. azteca* [10].

CASE STUDIES

Toxicity tests

The general toxicity test methods outlined in Appendix A for *H. azteca* and *C. tentans* have been used to successfully evaluate a variety of field-collected and contaminant-spiked

sediments. Most of the case studies described below were conducted at the EPA's Mid-Continent Ecology Division, Duluth, Minnesota. Consult methods manuals developed by the EPA [1] and ASTM [2] for details on the interlaboratory studies. The test methods evolved during the developmental phase; therefore, studies discussed below varied with respect to some test conditions. However, these variations were minor compared to the conditions outlined in Appendix A (e.g., >2 additions/d of overlying water, 1 vs. 1.5 ml of food) and would not be expected to modify water quality or exposure conditions substantially [9].

Ankley et al. [13] determined the toxicity of 17 field-collected sediments to *H. azteca* to evaluate the use of acid-volatile sulfide as a partitioning phase for predicting the bioavailability of cationic metals (Cd, Ni). Daily addition of overlying water was higher than that recommended in Appendix A (12 vs. 2 additions/d); however, the test was successfully completed with mean survival in a control sediment of 100%. Tests with *H. azteca* were also conducted with Cu-contaminated sediments from two systems. Concentrations of Cu in pore water were highly correlated to the toxicity of 22 test sediments to *H. azteca* [56]. As stated above, addition of overlying water was greater than that recommended in Appendix A (20 vs. 2 additions/d) and survival in the control sediment ranged from 90 to 100%. West et al. [27] also evaluated the toxicity of a number of samples from the Keweenaw Waterway. *Hyaella azteca* was the most sensitive to the lethal effects of test sediments, followed by *C. tentans* and then *L. variegatus*. Survival of the three test organisms in a control sediment was $\geq 90\%$.

Samples contaminated with a variety of pesticides and pesticide metabolites have been tested using methods similar to those described in Appendix A. Hoke et al. [57] and West et al. [58] determined the toxicity of field-collected sediments contaminated with DDT, DDE, and DDD to *H. azteca* and *C. tentans*. The objective of these studies was to evaluate the use of equilibrium partitioning as a method for developing sediment quality criteria for nonionic organic chemicals. Both studies confirmed the importance of organic carbon as a partitioning phase for predicting bioavailability of organochlorines to the invertebrates. Survival in the controls ranged from 80 to 100%. Ankley et al. [59] determined the toxicity of chlorpyrifos-spiked sediments to *C. tentans* to evaluate the possibility of deriving a sediment quality criteria for this organophosphate. Equilibrium calculations based on organic carbon partitioning were used to predict the bioavailability of the pesticide in a variety of sediments. Two different control sediments were spiked in these experiments; survival of organisms in the controls ranged from 83 to 93% and mean dry weight was 1.5 mg/organism after the 10-d exposure.

In another study that focused on the development and evaluation of sediment quality criteria, Hoke et al. [60] evaluated the toxicity of three sediments spiked with various concentrations of dieldrin to *H. azteca*, and toxicity of one spiked sediment to *C. tentans*. Control survival of *H. azteca* in the two control sediments was $\geq 85\%$; however, survival of amphipods in a third control sediment was variable over the course of the four different experiments, ranging from 63 to 85% (mean 76%). Mean survival of *C. tentans* in the

third control sediment was 100% and mean dry weight was 0.93 mg/organism.

Various experiments employing the basic test methods have also been conducted using uncontaminated field-collected sediments or quartz sand. Initial experiments with quartz sand [9] helped define the feeding rates recommended in Appendix A. These experiments also indicated that an addition of overlying water of 2 to 4 volumes/d maintained acceptable overlying water quality, while minimizing loss of contaminants from test sediments. Call et al. [61] used these methods to evaluate the importance of growth on emergence and reproductive success of *C. tentans*. Midge larvae were tested with a quartz-sand substrate and fed various quantities of Tetrafin. Midges receiving an amount of food similar to the rate recommended in Appendix A had a mean survival of 90% and a mean dry weight of 0.99 mg/organism after 10 d. Similar methods outlined in Appendix A were used successfully to conduct 28-d *H. azteca* tests with 49 uncontaminated sediments from the upper Mississippi River (F.J. Dwyer, unpublished data). Ankley et al. [28] tested up to 50 uncontaminated sediments from around the Great Lakes to evaluate the possible influence of natural physicochemical characteristics of sediment (e.g., grain size) on the response of *H. azteca* and *C. tentans*. In this study, about 80% of the *H. azteca* tests resulted in $\geq 80\%$ survival and 95% of the *C. tentans* tests resulted in $\geq 70\%$ survival. About 98% of the tests resulted in a mean *C. tentans* dry weight of ≥ 0.6 mg/organism after 10 d. Over the course of the study with Great Lakes sediments, 9 of 10 tests conducted with a control sediment resulted in acceptable control survival [28].

In summary, the toxicity test methods for *H. azteca* and *C. tentans* outlined in Appendices A and B have been used with a variety of sediments to address issues ranging from site assessment to the bioavailability of organic and inorganic contaminants in field-collected or spiked samples. Control survival of both organisms and growth (dry weight) of *C. tentans* have consistently met or exceeded the acceptability criteria listed in Appendix B. In addition, these methods have consistently resulted in acceptable quality of overlying water (e.g., dissolved oxygen, pH, and hardness). Thus, these case studies demonstrate the robustness of the methods for successfully conducting toxicity tests with both *H. azteca* and *C. tentans*.

Bioaccumulation tests

Methods for conducting bioaccumulation tests with *L. variegatus* have also varied slightly over the years; however, test conditions (e.g., test length, exposure systems) have been consistent enough for evaluation of the robustness of the guidance outlined in Appendix A. Ankley et al. [43] compared the bioaccumulation of PCBs by *L. variegatus* exposed in the laboratory to sediments from the lower Fox River-Green Bay to PCB residues in synoptic collections of oligochaetes from the field. Good agreement was observed between PCB concentrations in the laboratory and field organisms, particularly for those congeners with K_{ow} values < 7 . This suggests that for superhydrophobic chemicals, laboratory exposures longer than 28 d may be required to reach equilibrium. Good agreement in bioaccumulation between laboratory-exposed

L. variegatus and field-collected oligochaetes was also observed for sediments tested from the upper Mississippi River (E.L. Brunson, unpublished data). Concentrations of DDT reached 90% of steady state by day 14 of a 56-d test with *L. variegatus* exposed to field-collected sediments (E.L. Brunson, unpublished data). However, low molecular weight PAHs (i.e., acenaphthylene, fluorene, phenanthrene) generally peaked by day 3 and tended to decline to day 56. Concentrations of high molecular weight PAHs (i.e., benzo(b)fluoranthene, benzo(e)pyrene, indeno(1,2,3-c,d) pyrene) typically either peaked by day 28 or continued to increase during the 56-d exposure.

Bioaccumulation of contaminants by indigenous oligochaetes that were recovered from the same chamber with introduced *L. variegatus* in a 28-d test were also evaluated. Peak concentrations of select PAHs and DDT were similar in the indigenous oligochaetes and in *L. variegatus* exposed in the same chamber (E.L. Brunson, unpublished data).

Bioaccumulation of metals from sediments has also been evaluated using *L. variegatus*. Ankley et al. [13] reported elevated concentrations of Cd and Ni in worms after 10-d exposures to field-collected sediments where the metal (Cd + Ni):acid-volatile sulfide ratio exceeded 1, but not in samples where the ratio was <1. Ankley et al. [62] also found that worms did not bioaccumulate metals from three sediments containing elevated concentrations of Cd, Ni, Zn, Cu, and Pb, when there was sufficient acid-volatile sulfide to complex metals.

CONCLUSIONS

The methods outlined in Appendices A and B have been used successfully to evaluate the toxicity and bioaccumulation of contaminants associated with sediments. The procedures require limited special equipment and are relatively rapid, simple, and inexpensive to conduct. Use of these specific methods has several advantages, including increased precision and comparability of data as well as greater regulatory and legal acceptance. However, the use of specific methods may inhibit development of new procedures or result in incomplete characterization of effects resulting from the testing of specified conditions (e.g., only one test temperature). Although methods have described specific procedures for conducting sediment tests, research is continuing on several critical issues necessary for interpretation of test results, including the influence of light quality and quantity, feeding and nutritional requirements of the test organisms, performance criteria for organism health, chronic tests, and confirmation of laboratory-derived data with that of natural benthic populations. Results of these ongoing studies will be used to refine these methods further.

Sediment-assessment approaches can be classified as numeric (e.g., equilibrium partitioning), descriptive (e.g., whole-sediment toxicity tests), or a combination of numeric and descriptive approaches (e.g., Effects Range Median (ERM); [63]). Numeric methods can be used to derive chemical-specific sediment quality criteria. Descriptive methods such as toxicity tests with field-collected sediment cannot be used alone to develop numerical criteria for individual

chemicals. Alternatively, descriptive methods can be used to assess the impact of chemical mixtures in sediments, whereas it is difficult to predict the combined effects of contaminants from chemical-specific approaches. Although several approaches can be used to make site-specific decisions, no single approach can fully assess sediment quality. Overall, an integration of several methods using the weight of evidence is the most desirable means for assessing the effects of contaminants associated with sediment.

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APPENDIX A

Conditions for conducting sediment tests with *Hyalella azteca* (HA), *Chironomus tentans* (CT), and *Lumbriculus variegatus* (LV) as described in EPA [1] and ASTM [2,11].

Parameter	Conditions
1. Test type	Whole-sediment with renewal of overlying water
2. Temperature	23°C
3. Light quality	Wide-spectrum fluorescent lights
4. Illuminance	~500 to 1,000 lux
5. Photoperiod	16:8 h light:dark
6. Test chamber	HA/CT: 300-ml high-form lipless beaker LV: 4- to 6-L chamber
7. Sediment volume	HA/CT: 100 ml LV: 1 L or more depending on sediment organic carbon
8. Overlying water	HA/CT: 175 ml LV: 1 L or more depending on sediment organic carbon
9. Renewal water	2 volume additions/d
10. Age of organisms	HA: 7- to 14-d old CT: 3rd instar LV: adults
11. Organisms/chamber	HA/CT: 10 LV: sediment organic carbon:organism dry weight 50:1
12. Number replicates	8 for toxicity and 5 for bioaccumulation testing
13. Feeding	HA: YCT CT: Tetrafin® LV: not fed during test
14. Aeration	None if DO > 40% of saturation in overlying water
15. Overlying water	Culture, well, surface, site, or reconstituted water
16. Chamber cleaning	Gently brush screens on <i>outside</i> of chambers as needed
17. Water quality	Hardness, alkalinity, conductivity, pH, ammonia at start and end; temperature and dissolved oxygen daily

Appendix A continued

Parameter	Conditions
18. Test duration	HA/CT: 10 d LV: 28 d
19. End points	HA: survival (growth optional) CT: survival, growth (head capsule width optional) LV: bioaccumulation
20. Test acceptability	HA: minimum mean control survival of 80% and performance-based criteria (Appendix B) CT: minimum mean control survival of 70% and performance-based criteria (Appendix B) LV: number of organisms in a 4-d toxicity screening test should not be significantly reduced in the test sediment relative to the control sediment; test organisms should burrow into test sediment (Appendix B)

APPENDIX B

Recommended performance-based criteria for test acceptability as described in EPA [1] and ASTM [2,11].

Hyalella azteca 10-d toxicity test

1. Age at the start of the test must be between 7 to 14 d old.
2. Average survival in the control sediment must be $\geq 80\%$ at the end of the test.

Chironomus tentans 10-d toxicity test

1. At least 50% of the larvae must be in the 3rd instar at the start of the test (with the remaining larvae 2nd instar as determined by width of the head capsule).
2. Average survival in the control sediment must be $\geq 70\%$ at the end of the test.
3. Average size of organisms in the control sediment must be at least 0.6 mg at the end of the test.

Lumbriculus variegatus 28-d bioaccumulation test

1. Numbers in a 4-d toxicity screening test should not be significantly reduced in the test sediment relative to the control sediment.
2. Test organisms should burrow into test sediment. Avoidance of test sediment by *L. variegatus* may decrease bioaccumulation.

Culturing test organisms

1. Laboratories should perform monthly 96-h water-only reference-toxicity tests. If these tests are not conducted monthly, the lot of organisms used to start a sediment test must be evaluated using a reference toxicant.
2. Laboratories should track (a) parental survival of *H. azteca*, (b) time to first emergence for each culture for *C. tentans*, and (c) the frequency of populations doubling for *L. variegatus* cul-

tures. Records should also be kept on the frequency of restarting cultures.

3. Laboratories should record the following water quality characteristics of the cultures at least quarterly and the day before the start of a sediment test: (a) pH, (b) hardness, (c) alkalinity, and (d) ammonia. Dissolved oxygen should be measured weekly. Temperature should be recorded daily.
4. Laboratories should characterize and monitor background contamination and nutrient quality of food if problems are observed in culturing or testing organisms. Food used to culture organisms should be analyzed before the start of a test for compounds to be evaluated in the bioaccumulation test.
5. Physiological measurements such as lipid content might provide useful information regarding the health of the cultures.

Additional requirements

1. All organisms in a test must be from the same source.
2. It is desirable to start tests soon after collection of sediment from the field.
3. All test chambers (and compartments) should be identical and should contain the same amount of sediment and overlying water.
4. Negative-control sediment and appropriate solvent controls must be tested. Concentration of solvent must not adversely affect test organisms.
5. Test organisms must be cultured and tested at 23°C (daily mean test temperature $23 \pm 1^\circ\text{C}$ and instantaneous test temperature $23 \pm 3^\circ\text{C}$).
6. Hardness, alkalinity, pH, and ammonia in the water above the sediment within a treatment should not vary by more than 50% during the test.
7. Natural physicochemical characteristics of test sediment collected from the field should be within the tolerance limits of the test organisms.